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THE NUTRITION OF *PARANEMERTES PEREGRINA* (RHYNCHOCOELA: HOPLONEMERTEA). II. OBSERVATIONS ON THE STRUCTURE OF THE GUT AND PROBOSCIS, SITE AND SEQUENCE OF DIGESTION, AND FOOD RESERVES[1]

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The division of the Rhynchocoela into Anopla and Enopla is partly based upon the morphology of the gut and proboscis (Hyman, 1951). In anoplan nemerteans the mouth and proboscis pore are quite separate and the proboscis lacks stylet armature, whereas enoplans have the gut and proboscis opening anteriorly via a common rhynchodaeal aperture. In addition, the hoplonemertean enoplans have their proboscis armed by a characteristic stylet apparatus.

Despite these major differences, the basic digestive physiology of both groups is similar. It consists of an acidic extracellular proteolytic phase, followed by the phagocytosis of food particles and their subsequent intracellular digestion by means of proteases, carbohydrases and lipases acting in harmony (Jennings and Gibson, 1969). Intracellular digestion occurs in two stages, first acidic and secondly alkaline, with acid and alkaline phosphatases being associated with the respective phases.

An exception to this general rule is found in the atypical microphagous bdellonemerteans, where the loss of the carnivorous habit has resulted in a reduction of the emphasis placed upon proteolysis concurrent with an increase in the amount of carbohydrase activity at both the extra- and intracellular sites (Gibson and Jennings, 1969).

In all the hoplonemertean species so far investigated the endopeptidases responsible for extracellular proteolysis are produced and secreted by the gastrodermal columnar cells. Functional gland cells are present in the intestinal epithelium, but their precise role in the digestive processes has not yet been determined.

Greater variation in hoplonemerteans is, however, found in the acid-secreting mechanisms of the foregut, and two distinct types can be recognized (Jennings and Gibson, 1969). *Prostoma rubrum*, in common with anoplan species, possesses acidophilic gland cells which at all times exhibit demonstrable carbonic anhydrase activity, but amphiporids and tetrastemmids lack this enzyme completely although still producing acidic secretions. These variations in physiology may be attributed to differences in feeding, as *Amphiporus*, and probably *Tetrastemma* also, possesses a specialized feeding mechanism, in contrast to *Prostoma* which

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feeds in the manner more typical of the phylum as a whole (various authors, summarized by Jennings and Gibson, 1969).

The food reserves of nemerteans consist principally of fat globules stored in the gastrodermis, although some deposits on occasion can also be found in the general body parenchyma. No protein reserves have been recorded for the group, and the storage of glycogen appears to be of secondary importance. Glycogen occurs as tiny granules scattered throughout the gastrodermis, parenchyma and musculature, with occasional aggregations around the gonads (Reisinger, 1926; Jennings, 1960; Gibson and Jennings, 1969; Jennings and Gibson, 1969).

Until recently the common Pacific hoplonemertean *Paranemertes peregrina* Coe had been little investigated with respect to its nutrition. Roe (1967, 1970) has reported on the food and feeding behavior of this species, and the present study forms a logical sequence to this investigation as well as adding to our knowledge of the digestive physiology in nemerteans.

Materials and Methods

Specimens of *Paranemertes peregrina* were obtained from intertidal muddy shores at Garrison Bay and Snug Harbor, San Juan Island, Washington, during July and August 1969.

Histological studies on the structure of the gut and proboscis were made on specimens fixed in marine Bouin, Susa, or 10% neutral formalin containing 3% sodium chloride. Paraffin wax (56° C m.p.) sections cut at 6–8 μ were subsequently stained by routine methods, including hematoxylin and eosin, Feulgen, Mayer's hemalum, periodic acid-Schiff (PAS), Mallory's trichrome, 1% aqueous Alcian blue (for mucopolysaccharides), or by the bromphenol blue technique of Johri and Smith (1956) for proteins.

The site and sequence of digestion was determined from the examination of specimens fixed at progressive time intervals following an observed meal. In all cases the readily accepted nereid polychaete *Platynereis bicanaliculata*, collected from the same areas as *Paranemertes*, was used as the food.

Specimens were fixed for 2–4 h at 4° C in 10% formalin containing 3% sodium chloride, phosphate-buffered to pH 7.0. Following fixation they were either washed in ice-cold distilled water and sectioned directly on an International Harris Model CTD cryostat, or dehydrated through graded acetones at 4° C, cleared in xylene at 18–22° C, and infiltrated *in vacuo* in paraffin wax of melting point 45° C. Paraffin sections were mounted on albumenized slides, air dried at room temperature, and dewaxed before rinsing in absolute acetone prior to incubation for enzyme visualization. Cryostat sections were similarly subjected to acetone treatment in order to remove fats before being incubated. All dehydration, clearing and infiltration times were kept to a minimum suitable to the size of the specimens being processed.

The following methods were used in the investigation of enzymes present: the Hausler (1958) method for carbonic anhydrase; the Hess and Pearse (1958) indoxyl acetate method for cathepsin-C type endopeptidases, as used by Jennings (1962a, 1962b), Rosenbaum and Ditzion (1963), Jennings and Mettrick (1968), Jennings and Gibson (1969), and Jennings and Gelder (1969); the Burstone and Folk (1956) L-leucyl-β-naphthylamide technique for exopeptidases of the leucine-

aminopeptidase type; the indoxyl acetate (Holt, 1958) and α-naphthyl acetate (Gomori, 1952) methods for non-specific esterases; the Gomori (1952) Tween 80 method for lipase; the Burstone (1958) azo-dye technique for acid phosphatase; and calcium salt method (Gomori, 1939) for alkaline phosphatase.

Controls used for these histochemical methods included heat-inactivated sections and media from which the specific substrate had been omitted.

The distribution of the food reserves was studied in paraffin sections of specimens fixed either in Flemming's osmium tetroxide fluid (for fats), or 90% alcohol containing 1% picric acid and subsequently stained by the PAS or Bauer methods (for glycogen).

Observations

Structure of the gut and proboscis

As in other monostyliferous hoplonemerteans, the gut and proboscis of *Paranemertes* lack separate external openings. A single anterior pore, the rhynchodaeal aperture, opens from the anterior tip into a somewhat cone-shaped chamber, the rhynchodaeum, and it is from the back of this that the proboscis and gut open dorsally and ventrally respectively (Fig. 1).

The rhynchodaeal epithelium possesses no gland cells and consists only of ciliated cuboidal cells 6–9 μ tall overlying a thin basophilic basement membrane. In common with the remainder of the gut, the rhynchodeum has no specific musculature associated with it, although numerous obliquely arranged fibers are embedded in the surrounding parenchyma. In most specimens local aggregation of muscle fibers at the junction of proboscis and rhynchodeum is suggestive of a possible sphincter.

Around the rhynchodaeum can also be found the lobular frontal glands, which extend posteriorly to just behind the cerebral ganglia, and the paired cerebral organs, which open anteriorly into the cephalic slits.

The gut is divisible histologically into two distinct regions, the foregut and the intestine. Unlike the situation reported for many other hoplonemertean species, *Paranemertes* does not possess either a distinct esophagus or a pyloric tube, and the foregut opens directly into the rhynchodeum and intestine at the appropriate points. The intestine extends ventrally and anteriorly as a blind-ending cecum, both intestine and cecum bearing numerous, often long, multilobed diverticula.

The foregut epithelium is folded and glandular, and consists of two distinct cell types. The principal components are columnar cells 45–60 μ tall and 6–8 μ wide bearing dense distal cilia 4–5 μ long. These cells are filled with a coarsely granular basophilic cytoplasm that fails to react to histochemical stains for either mucus or protein. Their single oval or spherical nucleus, 3–4.5 μ in diameter, is situated proximally. Evidence of cytoplasmic vacuolation can frequently be found, particularly in recently fed specimens.

Between the basophilic cells are non-ciliated pyriform gland cells of similar height but only 4–6 μ width, packed with acidophilic proteinaceous spheres of 1 μ or less diameter. Gland cell nuclei, 4–5 μ long and 2–2.5 μ wide, are positioned proximally with their long axes approximately at right angles to the epithelial basement membrane.

Both cell types are secretory when fully developed. The basophils extrude their contents to the foregut lumen between their distal cilia, but the acidophils open either directly to the lumen or discharge their spheres via secretory tracts extending between the columnar cells. Shortly after ingestion is commenced, evidence of secretion can be found in the foregut lumen, the basophilic component appearing as finely particulate and irregular "strings," the acidophilic spheres complete and isolated. There is some evidence to suggest that the acidophilic secretions initially expand after discharge, and then rupture to release their contents into the lumen.

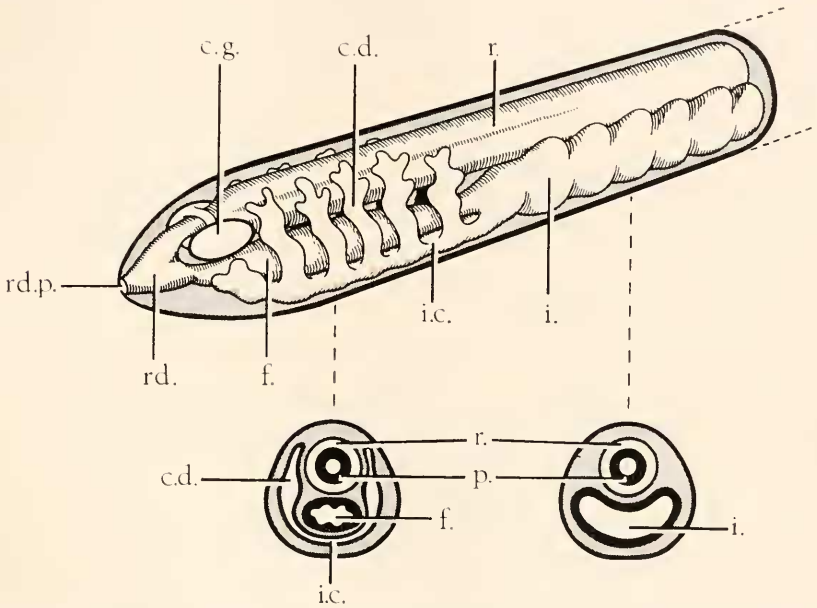

FIGURE 1. *Paranemertes peregrina.* Stereogrammatic representation to show the relative positions of the proboscis and alimentary canal, characteristic of this type of hoplonemertean. The appearance of the body in transverse section is shown for two points in the lower diagrams; c.d., cecal diverticulum; c.g., cerebral ganglion; f., foregut; i., intestine; i.c., intestinal cecum; p., proboscis; r., rhynchocoel; rd., rhynchodeum; rd.p., rhynchodeal pore.

The distribution of the adipophilic glands in the foregut is such that they are concentrated in the anterior half where they and the columnar cells occur in approximately equal numbers. A short region immediately adjoining the rhynchodeum tends to lack these glands, however, and it is probable that this portion of the foregut is equivalent in the esophagus described for other hoplonemertean species. The posterior foregut epithelium shows a progressive decrease in the density of the acidophils as the intestine is approached, and in the region just anterior to the hindgut the ratio of gland to columnar cells is 1:20 or more.

A distinct pyloric tube is absent from *Paranemertes* and there is no decrease in epithelial height although the foregut tends to be less folded. At the junction of foregut and intestine a loose aggregation of circular and oblique muscle fibers is found in the surrounding parenchyma. These may serve as sphincter muscles to

this part of the gut, although such an arrangement was not seen in all specimens examined.

The intestinal wall, or gastrodermis, is in its structure very similar to that described for other species. It consists of acidophilic pyriform gland cells interspersed between ciliated columnar cells. In starved specimens the columnar cells are 60–80 μ tall and 6–8 μ wide, their sparsely distributed distal cilia extending 12–14 μ into the intestinal lumen. Subspherical nuclei 2–2.5 μ in diameter are embedded proximally in the cytoplasm, the latter being finely particulate and possessing no particular staining affinities. The proximal regions of the columnar cells also contain variable numbers of acidophilic proteinaceous inclusions up to 3–4 μ diameter, and these react positively to the Hess and Pearse technique for endopeptidases.

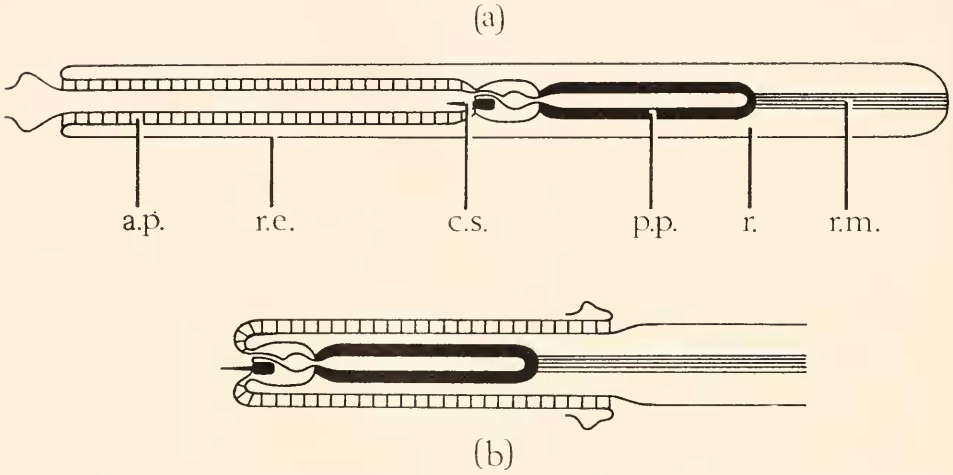

FIGURE 2. *Paranemertes peregrina.* Diagram to show the relationships between the parts of the proboscis in the retracted (a) and protruded (b) positions. Note how in (b) the central stylet is terminal; a.p., anterior proboscis epithelium; c.s., central stylet positioned in muscular bulb; p.p., posterior proboscis epithelium; r., rhynchocoel; r.e., rhynchocoel endothelium; r.m., proboscis retractor muscle.

In contrast, the narrower gland cells, filled with acidophilic proteinaceous spheres of maximum diameter 1.5 μ, fail to react to histochemical methods for either endopeptidases or non-specific esterases, although their contents are discharged into the intestinal lumen and clearly play some part in the extracellular digestive processes.

Gland cells are most numerous in the anterior intestine and cecum, their numbers decreasing posteriorly so that they are almost absent from the region near the anus. The ratios of gland to columnar cells are about 1:1 and 1:30, respectively.

The proboscis of *Paranemertes* is armed by a single, needle-shaped, central stylet, and lies coiled in a rhynchocoel which extends for only about one-quarter of this body length. The rhynchocoel is lined by a thin endothelium overlying muscle layers comprised of inner longitudinal and outer circular fibers.

Three distinct regions of the proboscis can be recognized. In the retracted position (Fig. 2a) these are an anterior thick-walled tube, a short central muscular bulb housing the stylet apparatus, and a posterior acidophilic portion whose rearmost extremity is connected to the rhynchocoel by the proboscis retractor muscle.

The anterior proboscis epithelium is composed of two cell types arranged in a distinctive manner (Fig. 3). Elongate pyriform gland cells packed with proteinaceous acidophilic spheres of less than 1 μ diameter are interspersed with irregular-shaped columnar cells whose distal regions are filled with finely particulate, nonproteinaceous basophilic cytoplasm. In the retracted position the cellular arrangement is somewhat obscured (Fig. 4), but when the proboscis is protruded the epithelium forms definite papillae, each papilla consisting of a series of

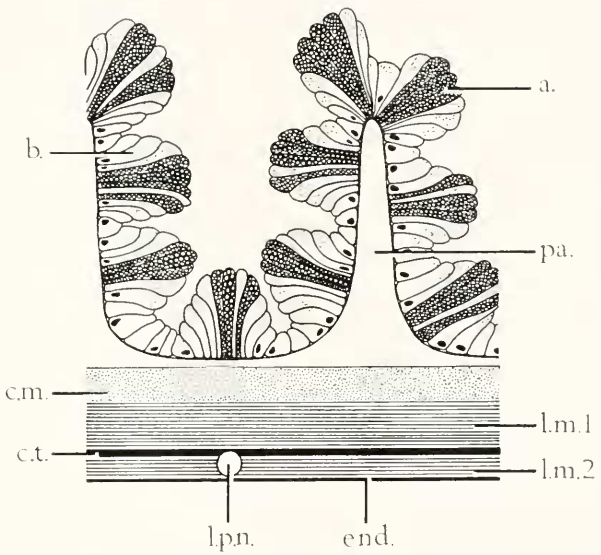

FIGURE 3. *Paranemertes peregrina*. Diagrammatic representation of the structure of the anterior proboscis when in the protruded position; a., acidophilic gland cell; b., basophilic columnar cell; c.m., circular muscle layer; c.t., connective tissue; end., endothelium; l.m.1, inner longitudinal muscle layer; l.m.2, outer longitudinal muscle layer; l.p.n., longitudinal proboscis nerve; pa., parenchyma.

groups of acidophilic cells flanked by columnal cells. The overall epithelial height at this time is approximately 40–50 μ, with papillae extending outward for 0.1–0.12 mm (Fig. 3). Both cell types discharge their contents when the proboscis is in use, their secretions showing a similar appearance to those described for the foregut.

Beneath the epithelium parenchymatous tissue forms a layer that extends into the papillae to form a semi-rigid core. The parenchyma overlies three muscle zones consisting of a single layer of circular fibers and two outer layers of longitudinal fibers. The longitudinal muscles are separated by a narrow zone of connective tissue. Enclosing the anterior proboscis a thin endothelium lies next to the outer longitudinal muscle layer.

The proboscis of *Paranemertes* is furnished with fourteen longitudinal nerves that extend the full length of the anterior region in the outermost muscle layer and its adjoining connective tissue.

At its posterior end the anterior proboscis narrows into the central muscular bulb, which is divided into two parts. The anterior region contains the stylet apparatus. The single central stylet, 80 μ long, is carried on a cylindrical waisted base of about 100 μ length and 50 μ maximum diameter. On either side of, and slightly anterior to, the central stylet are the paired accessory stylet pouches, each containing from two to eight accessory stylets of similar size to the central one. Both central and accessory stylets show the braided or fluted effect described by Coe (1905) for the more southerly Californian variety of *Paranemertes peregrina*.

The posterior half of the stylet bulb consists entirely of muscle fibers arranged around a sac-like central lumen, and it is probably through the contractions of these muscles that the proboscis secretions are forced into the body of the prey via the stylet wounds. The bulb lumen connects the anterior and posterior proboscis chambers by narrow canals (Fig. 2).

The third, posterior, region of the proboscis consists of a thin endothelium enclosing a single muscle layer of longitudinal and oblique fibers. Bordering the musculature a parenchymatous layer lies below the inner epithelium consisting of interstitial and gland cells that are not arranged into papillae. Glands, 15–20 μ long and 5–8 μ wide, are filled with irregular-shaped proteinaceous acidophilic granules of maximum dimension about 1.5 μ. There are no basophilic components in this proboscis region, and the interstitial cells possess no particular staining affinities. The proboscis lumen is often partly filled with a coarsely granular matrix that is secreted by the gland cells, and soon after proboscis eversion has taken place the lumenar contents show an increase in density at the same time as the glands can be seen discharging their contents.

The functions of the proboscis

It seems evident that the two major proboscis regions possess distinctive roles in the function of the organ. When the proboscis is protruded the central stylet is terminal (Roe, 1967), and any secretions poured into the wound inflicted by the stylet, forced from the proboscis by the contractions of the muscular bulb, can only arise from the posterior gland cells (Fig. 2b). Immobilization of nereid prey does not occur in the absence of stylet penetration, and it must therefore be presumed that the paralytic toxins are produced and secreted only by the posterior proboscis epithelium. Any comparable substances discharged from the anterior proboscis papillae would be both distant to the stylet wound, and subject to dilution from the surrounding sea water. However, these theoretical considerations are at variance with the findings of W. R. Kem, University of Illinois, unpublished results, who reports that the anabaseine toxin comprises some 7% of the wet tissue weight of the anterior proboscis, as well as being secreted by the general epidermis. This author fails to comment on the distribution of the toxin in the posterior proboscis and, in the light of this conflicting evidence, it seems inadvisable to draw further conclusions until additional investigations have been conducted.

The papilliary secretions, as noted earlier, closely resemble in appearance those of the foregut. The structure of the anterior proboscis suggests that the papillae assist in the organ gripping its catch, and it is likely that some of the secretions at least are viscous and play a supplementary role. Both non-specific esterase and acid phosphatase activity have been recorded in the acidophilic gland cells, with the former appearing additionally in the papilliary secretions. Since the basophilic secretions are distinctly "stringy" in appearance, it may be suggested that it is these that comprise the viscous component of the papilliary products, and that the gland cell esterases possess other roles. The precise function of the enzymes has not been established, but they may be involved in either initiating the disruption of the polychaete epidermis or in enhancing the viscous properties of the basophilic secretions. There is no evidence to suggest that *Paranemertes* possesses an extracorporeal digestive phase, as reported for other hoplonemertean species by Jennings and Gibson (1969).

The site and sequence of digestion

The food and feeding mechanism of *Paranemertes* have been fully reported by Roe (1967). Nereid polychaetes are caught by the proboscis, immobilized by its secretions, and ingested whole by means of a sucking action. At this time the foregut actively discharges its secretions, which have an acidic pH value of 5.5–6.0, as determined from *Platynereis* specimens examined during ingestion after having been previously stained with indicator dyes. No carbonic anhydrase activity was ever observed in any part of the gut, so the acid secreting mechanisms must involve other enzymes, presumably associated with the proteinaceous acidophilic glands. The role of the foregut basophilic secretions is not clearly understood, but two possibilities may be suggested. First, they may possess a lubricative function to facilitate ingestion or, second, they may activate the acidophilic secretions within the gut lumen.

Food material thus enters the intestine in an acidic medium, the acid secretions serving both to kill the prey and provide the appropriate pH level for subsequent extracellular proteolysis. As the food enters the intestine the gastrodermal gland cells discharge their contents. At the same time there is an increase in the number of the acidophilic inclusions within the columnar cells and they are discharged into the gut lumen between the cilia. As noted earlier, these inclusions contain cathepsin-C like endopeptidases (Fig. 5) and the amount of intralumenar activity of these enzymes increases as more of the inclusions are discharged.

Within two hours of a meal the food is sufficiently broken up to allow the phagocytosis of food particles by the columnar cells, food vacuoles formed at this time showing strong endopeptidase activity. There is no evidence to suggest that endopeptidases are secreted cytoplasmically, so that the intracellular activity observed presumably originated from the gut lumen, being taken into the cells during the phagocytotic processes. During this digestive phase strong acid phosphatase activity can be demonstrated in and around the food vacuoles (Fig. 6), where it is presumed to be concerned in some way with the intravacuolar maintenance of the acidic pH necessary for the efficient functioning of the proteases.

As time progresses the number of food vacuoles increases and six hours after a meal the columnar cells are packed with them. The early phase of intracellular

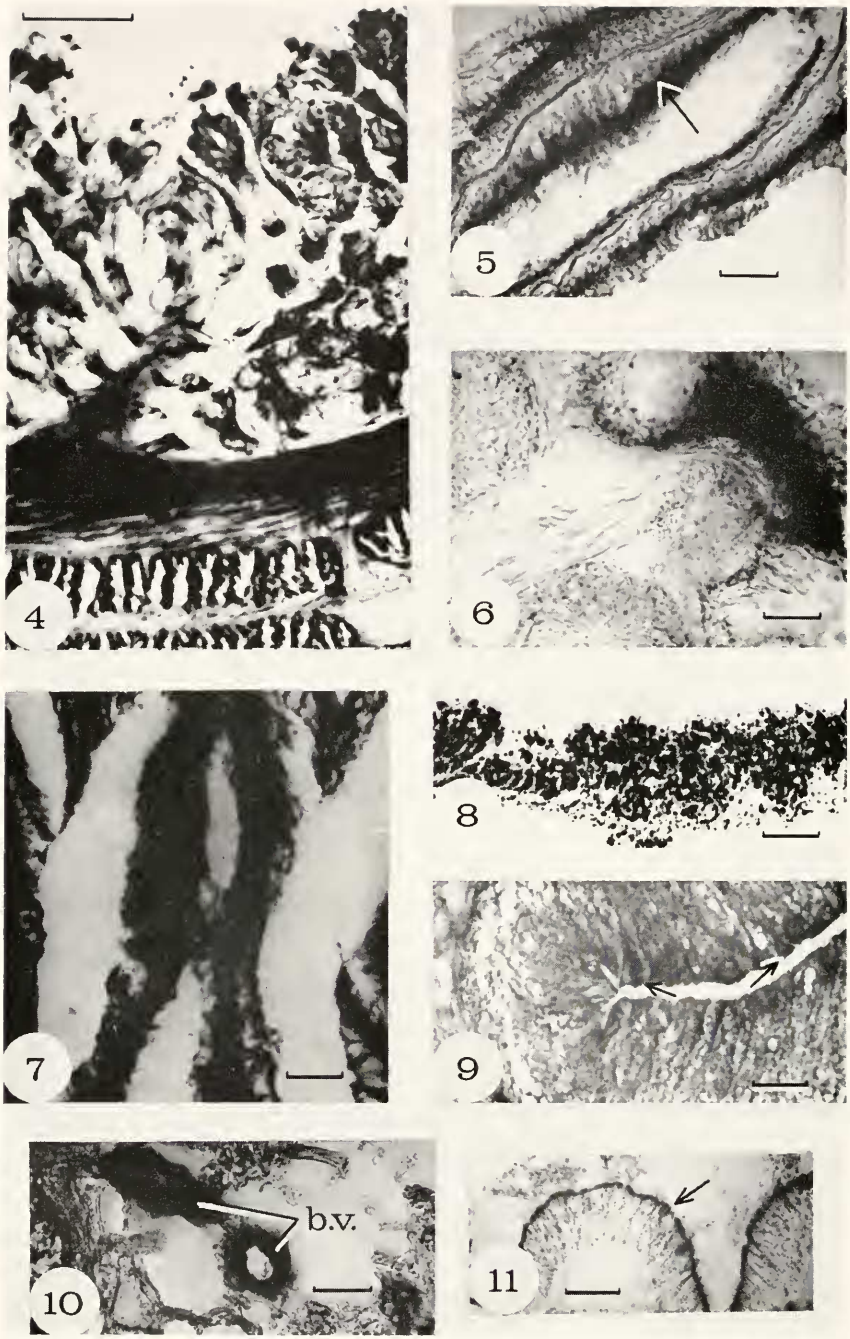

FIGURE 4. *Paranemertes peregrina.* Transverse section through the anterior proboscis in retracted position, showing how the cellular arrangement of the papillae is obscured; compare with Figure 3; Mallory; scale: 20 μ.

digestion lasts for up to thirty-six hours or more, by which time the residues of the meal have been evacuated from the gut lumen. Following this there is a decline in the amount of demonstrable acid phosphatase and endopeptidase activity, these enzymes becoming replaced by exopeptidases and alkaline phosphatase at the same sites. The activity of these enzymes persists within the columnar cells until digestion is completed, when the exopeptidases disappear and cannot be further demonstrated until the appropriate stage of a subsequent meal. This is the usual pattern of exopeptidase activity as reported for other nemertean species. Alkaline phosphatase activity, which is intense during peak exopeptidase visualization (Fig. 7), is at all other times present only as a faint zone of activity at the distal margins of the columnar cells. The enzymes responsible for the second, alkaline stage of intracellular digestion can be demonstrated within food vacuoles and their surrounding cytoplasm for as long as ninety-six hours after a meal.

Carbohydrases and lipases were not demonstrated at any stage in digestion, but the failure to visualize their activity could be explained by the relatively low amounts present. Lipases, for example, have only been successfully demonstrated in specimens fed on a high fat diet (Jennings, 1962a), and carbohydrases have been reported only by inference (Jennings and Gibson, 1969). There is no reason to suppose that *Paranemertes* in any way differs with respect to its carbohydrate- and fat-digesting enzymes.

The food reserves

Fat forms the principal food reserve in *Paranemertes,* being stored mainly in the gastrodermal columnar cells as globules of 2 μ or less diameter (Fig. 8). Occasional deposits up to 7 μ across can be found, but these are irregularly distributed. Fat is deposited more or less uniformly throughout the cell cytoplasm, but tends to be absent from a distal zone 5–8 μ deep.

No fat deposits were found in the parenchyma, epidermis, foregut, blood system or body musculature, but occasional globules of 1.5–2 μ diameter were observed

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FIGURE 5. *Paranemertes peregrina.* Section through the gastrodermis of a starved specimen showing columnar cells with the acidophilic spheres which are the sites of endopeptidase activity (arrowed); Hess and Pearse method; scale: 60 μ.

FIGURE 6. *Paranemertes peregrina.* Part of the gastrodermis six hours after a meal showing the distribution of acid phosphatase activity (black) in and around food vacuoles; Burstone's azo dye method; scale: 30 μ.

FIGURE 7. *Paranemertes peregrina.* Oblique section through the gastrodermis during the later stages of intracellular digestion to show the intense alkaline phosphatase activity (black) distributed throughout the columnar cell cytoplasm and in food vacuoles; Gomori's calcium salt method; scale: 60 μ.

FIGURE 8. *Paranemertes peregrina.* Longitudinal section through part of the gastrodermis to show the distribution of fat globules; Flemming; scale: 12 μ.

FIGURE 9. *Paranemertes peregrina.* Transverse section of a part of the gastrodermis showing the restriction of glycogen storage to the distal regions of the columnar cells (arrowed); Bauer; scale: 20 μ.

FIGURE 10. *Paranemertes peregrina.* Section through two blood vessels (b.v.) to show the intense "leucine aminopeptidase" activity consistently present in their lining walls; Burstone and Folk method; scale: 10 μ.

FIGURE 11. *Paranemertes peregrina.* Longitudinal section through the body wall showing the narrow distal zone of non-specific esterase activity (arrowed); Gomori's -naphthyl acetate method; scale: 20 μ.

in the anterior proboscis, where they were restricted to the connective tissue separating the two layers of longitudinal muscle fibers.

At the time of collection mature specimens were not available, so no observations on fat deposition within the ova and ovarian endothelia can be made. These sites usually contain large amounts of fat in other species.

Small amounts of glycogen, occurring as tiny scattered granules, are stored in the distal regions of the gastrodermal columnar cells (Fig. 9), the body wall muscles (particularly the longitudinal layers), and in the parenchyma adjoining the gastrodermis.

Other sites of enzymic activity

Strong exopeptidase activity is consistently present in association with the blood vascular system (Fig. 10), a regular site for this enzyme previously reported in other species by Gibson and Jennings (1967).

Variable amounts of non-specific esterase activity were found in several other sites in the body, demonstrable by both the indoxyl acetate and α-naphthyl acetate techniques. At each site the intensity of activity is independent of the nutritive state.

Weak esterase activity was observed in the rhynchocoel endothelium, longitudinal body musculature, and endothelium and plasma of parts of the blood vascular system. Irregular, but stronger amounts were visualized in the connective tissue separating the two longitudinal muscle layers of the anterior proboscis, and in the outer sheath of parts of the main lateral nerve rords. The strongest esterase activity appeared in the tracts of the cerebral glands, and as a 2.5–4 μ thick distal border to the epidermis (Fig. 11).

The only other enzymic activity demonstrated was acid phosphatase, parallelling the non-specific esterase distribution in the epidermis.

Discussion

The digestive physiology of *Paranemertes peregrina* closely resembles that described for other nemertean species by Jennings and Gibson (1969). These authors showed that amongst hoplonemerteans interspecific differences occur principally with respect to the acid-secreting mechanisms of the foregut, specifically in the presence or absence of demonstrable carbonic anhydrase activity in the acidophilic gland cells. The occurrence of this enzyme appeared to be restricted to those species in which direct ingestion of the food was not preceded by an extracorporeal digestive phase, this being true for palaeo- and heteronemerteans also. *Paranemertes* thus differs from most other nemerteans in lacking both extracorporeal digestion and demonstrable carbonic anhydrase activity, although its foregut secretions are clearly acidic in nature. A similar situation is found in the bdellonemertean *Malacobdella grossa,* but physiological and morphological differences in this species can be related entirely to its atypical commensal habits (Gibson and Jennings, 1969).

Paranemertes is less closely related to *Amphiporus* and *Tetrastemma,* which lack carbonic anhydrase, than is *Prostoma,* which possesses the enzyme. This suggests that amongst these species variations in foregut physiology cannot

simply be related either to systematic position or feeding mechanism, and it can be concluded only that this aspect of hoplonemertean digestive physiology is subject to interspecific alteration whose controlling factors are not yet understood.

The absence of a distinct esophagus and pyloric tube from *Paranemertes* is much more likely to be related to the manner of feeding. A similar absence of foregut differentiation is found in anoplan species where the food is ingested directly in a manner like that described for *Paranemertes* by Roe (1967, 1970). In contrast, hoplonemertean species such as *Amphiporus*, in which the foregut is partially protruded for feeding, possess not only a distinct oesophagus and an extended pyloric tube, but also show much more folding of the foregut epithelium than is found in species feeding in the more conventional nemertean manner.

As in other carnivorous nemerteans, the emphasis in *Paranemertes* is placed upon the production of proteolytic enzymes by the gut, those acting extracellularly being secreted by the gastrodermal columnar cells in the usual hoplonemertean fashion. The gland cells also discharge their products into the intestinal lumen, but the nature of their secretions remains undetermined. It is possible that they represent additional proteolytic enzymes not demonstrable by the techniques employed in the present work.

The persistent zone of alkaline phosphatase activity in the gastrodermal border may be involved in the phagocytosis and absorption of food material from the gut lumen. These enzymes have been linked with the phosphorylative transfer of extracellular substances by Danielli (1952) and Erasmus (1957), and Halton (1967) has reported that in polyopisthocotylean Monogenea alkaline phosphatases are found distally in the gastrodermis during the absorption of food materials. Similar roles have been attributed to these enzymes in other nemerteans (Jennings, 1962a; Jennings and Gibson, 1969; Gibson and Jennings, 1969), in archiannelids (Jennings and Gelder, 1969), and in rodents (Hugon and Borgers, 1968). The enzymes may further be concerned with the uptake of lipids, since Noma (1964) and Raghavan and Ganguly (1967) have demonstrated that shortly after a meal and during the active absorption of materials there is an increase in the phospholipid content of the intestinal mucosa.

Of the enzymes found at sites other than in the gut, exopeptidases consistently present in the blood vascular system are believed to be involved in the circulation of amino acids and simple peptides, as discussed by Gibson and Jennings (1967).

The role of the epidermal enzymes has not been established, but they may be concerned in one or both of two distinct mechanisms. W. R. Kem (unpublished) recorded that 70% of the species' toxin is localized within the epidermal tissues, and the enzymes may thus play a part in the secretion of this substance as a defensive mechanism against predation. Distasteful epidermal secretions at least are apparently produced by other species, since Gibson (1968) recorded that the extremely voracious littoral fish *Parenophrys bubalis* consistently refused to feed on lineids, even when starved and readily prepared to accept a wide variety of other natural and artificial foods.

A somewhat more plausible explanation of the role of these enzymes concerns the uptake of simple nutrient materials from the environment. Fisher and Cramer (1967) showed that glucose and amino acids were absorbed across the epidermis of *Lineus ruber*, and concluded that the epidermal microvilli were involved in this process. Jennings and Gibson (1969), in reporting the occur-

rence of epidermal enzymic activity in a number of nemertean species, suggested that these enzymes may be concerned with the extracorporeal digestion of simple proteins or polypeptides, which could then be absorbed across the microvilli. This postulated link between epidermal enzymes, microvilli and absorption is further discussed by Jennings (1969), who comments on the similarity between gastrodermal and epidermal microvilli, although noting that the former are not apparently concerned in the normal phagocytotic processes of the gut. The epidermal absorption of nutrient materials may thus be a general feature of nemerteans, in which case the blood system exopeptidases may well be additionally involved in this mechanism.

The roles of other non-digestive enzymes are far from being understood. In the anterior proboscis connective tissue an association between the non-specific esterases and fat reserves may tentatively be drawn, as certain esterases are involved in the hydrolysis of triglycerides (Reid and Dunnill, 1969), and are believed to be partly responsible for intracellular lipid metabolism in bdellonemerteans (Gibson and Jennings, 1969). The absence of demonstrable lipases from these sites is, however, at variance with this suggestion.

Whether a similar role can be envisaged for esterases in other tissues is in doubt, particularly since fat deposits are otherwise restricted to the gastrodermis. Certainly in the cerebral glands, where strong esterase activity is persistently recorded, the enzymes must function in other metabolic processes. It is not known for certain whether these glands are involved with chemotactic (Reisinger, 1926), endocrine (Scharrer, 1941) or other functions.

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Summary

1. Digestion in the hoplonemertean *Paranemertes peregrina* is achieved by a combination of extra- and intracellular processes. The extracellular phase, effected in an acidic medium, involves endopeptidases secreted by the gastrodermal columnar cells, and other, as yet unidentified, substances discharged from the intestinal gland cells. The semi-digested food is then phagocytosed and digestion completed intracellularly by peptidases, carbohydrases and lipases acting in harmony. Intracellular digestion is initially acid and then alkaline, with acid and alkaline phosphatases associated with the appropriate phases.

2. Nereids used as food are caught by the proboscis, and immobilized by secretions produced by the posterior proboscis gland cells. These secretions are pumped into the body of the prey via wounds caused by the central stylet. The nature of these secretions has not definitely been established, but they may contain the toxin anabaseine.

3. The anterior proboscis secretions are concerned with aiding the grip of the proboscis papillae and possibly with initiating the denaturation of the prey epidermis.

4. Acid secretions are produced by the foregut via a mechanism that does not involve carbonic anhydrase.

5. Other sites of enzymic activity have been reported, and where possible suggestions made as to their probable roles.

6. Fat forms the principal food reserve, with major deposits being stored in the gastrodermal columnar cells, but some glycogen is stored in a variety of body tissues.

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